(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
15 May 2003 (15.05.2003)

PCT

(10) International Publication Number
WO 03/040398 A2

(51) International Patent Classification[7]: C12Q

(21) International Application Number: PCT/US02/34752

(22) International Filing Date: 30 October 2002 (30.10.2002)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
10/012,667 30 October 2001 (30.10.2001) US

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(81) Designated States *(national)*: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, OM, PH, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZM, ZW.

(84) Designated States *(regional)*: ARIPO patent (GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, SK, TR), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

Published:
— *without international search report and to be republished upon receipt of that report*

For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

WO 03/040398 A2

(54) Title: PROTEINS STABILIZED WITH POLYSACCHARIDE GUMS

(57) Abstract: Described are heat stable aqueous solutions or gels comprising a biologically effective amount of a protein and an effective stabilizing amount of a polysaccharide gum as well as heat stable solutions or gels suitable for use in an implantable drug delivery device at body temperature. Also disclosed are lyophilized compositions having biologically activity, where such lyophilized compositions are formed by lyophilozing the stabilized solutions or gels of the invention. Such lyophilized powders can be used after reconstitution with an amount of aqueous that provide an effective stabilizing concentration of polysaccharide and a pharmaceutically acceptable amount of therapeutic protein particularly against thermal and oxidative stress.

TITLE OF THE INVENTION

PROTEINS STABILIZED WITH POLYSACCHARIDE GUMS

This application claims the benefit of U.S. Application No. 10/012,667 filed October 30, 2001, the content of which is incorporated herein by reference as if completely rewritten herein.

TECHNICAL FIELD

The present invention relates to a heat stable aqueous solution or gel comprising a biologically effective amount of a protein and an effective stabilizing amount of a polysaccharide gum as well as heat stable solutions or gels suitable for use in an implantable drug delivery device. This invention also relates to lyophilized compositions having biological activity, where such lyophilized compositions are formed by lyophilizing the stabilized solutions or gels of the invention as well as the lyophilized compositions that are reconstituted in an aqueous buffer so as to provide a high concentration of polysaccharides that will stabilize proteins under physiological conditions.

BACKGROUND OF THE INVENTION

The commercial market for recombinant protein biopharmaceuticals is expanding rapidly as various biotechnology and pharmaceutical companies develop and test biologically active proteins. The emerging field of proteomics will likely provide protein targets useful for drug development, thereby enabling the market for recombinant protein biopharmaceuticals to continue its expansion.

Currently, proteins are utilized in a variety of diagnostic and therapeutic applications. For example, one protein used in a diagnostic application is the enzyme glucose oxidase, which is used in glucose assays. The hormone insulin is an example

of a protein utilized in therapeutic applications. However, proteins are particularly sensitive to certain environmental conditions and may not be stable at elevated temperatures, including physiological temperature of 37°C, in non-optimal aqueous solvent systems, or in organic solvent systems. Protein stability may also be affected by pH and buffer conditions and exposure to shear forces or other physical forces.

The stability of a protein refers to both its conformational stability, which is reflected in the protein's three-dimensional structure, and its chemical stability, which refers to the chemical composition of the protein's constituent amino acids. Protein instability can result in a marked decrease or complete loss of a protein's biological activity. Deleterious stresses such as organic solvents, extremes of pH, high temperatures, and/or dehydration (drying) can affect both the conformational and chemical stability of a protein. Chemical instability can result from processes such as (a) deamidation of the amino acids residues asparagine or glutamine, (b) oxidation of cysteine or methionine amino acid residues, or (c) cleavage at any of the peptide amide linkages of the protein. Examples of conformational instability include aggregation (fibrillation), precipitation, and subunit dissociation.

Because an inactive protein is useless, and in some cases deleterious, for most diagnostic and therapeutic applications, there is a need for a means by which proteins can be stabilized in solution at elevated temperatures (e.g. at and above room temperature, at body temperature or higher. It is known in the art that proteins can be stabilized in solution by the addition of soluble excipients that stabilize the monomeric, correctly folded protein conformation. Disaccharides such as trehalose, sucrose, or lactose, and surface active agents such as phospholipids, Tween, and Triton are examples of excipients useful for stabilizing proteins. These stabilizers must be used in non-toxic levels because in the case of therapeutic proteins, the stabilizers are necessarily administered to the patient with the protein.

U.S. Pat. No. 5,834,273 issued to Futatsugi et al. on November 10, 1998 provides a heat and protease resistant enzyme with improved storage stability. This enzyme is modified with a polysaccharide, polyamino acid, or synthetic polymer having a plurality of carboxyl groups by means of a crosslinking agent capable of binding both carboxyl groups and amino groups.

U.S. Pat. No. 5,736,625 issued to Callstrom et al. on April 7, 1998 discloses a method for preparing water soluble, saccharide-linked protein polymer conjugates that stabilize the protein in a hostile environment. The claimed method includes covalently binding the polymer to the protein through at least three linkers, each linker having three or more hydroxyl groups. The protein is conjugated at lysines or arginines.

U.S. Pat. No. 5,691,154 issued to Callstrom et al. on November 25, 1997 provides an enzyme linked immunoassay in which the enzyme is in the form of a water soluble polymer saccharide conjugate which is stable in hostile environments. The conjugate includes the enzyme which is linked to the polymer at multiple points through saccharide linker groups.

U.S. Pat. No. 5,612,053 issued to Baichwal et al. on March 18, 1997 discloses an inhalable powder formulation which includes cohesive composites of particles containing a medicament and a controlled release carrier which includes one or more polysaccharide gums of natural origin.

U.S. Pat. No. 5,492,821 issued to Callstrom et al. on February 20, 1996 discloses water soluble protein polymer conjugates in which proteins linked to an acrylic polymer at multiple points by means of saccharide linker groups. These conjugates are also stable in hostile environments.

U.S. Pat. No. 5,128,143 issued to Baichwal et al. on July 7, 1992 provides, for oral delivery, a slow release pharmaceutical excipient of an inert diluent and a hydrophilic material including xanthan gum and a galactomannan gum capable of cross-linking the xanthan gum in the presence of aqueous solutions.

Ispas-Szabo et al. demonstrated that the ability of starch tablets to swell and release low molecular weight drugs could be controlled by the degree that the starch was cross-linked. No data related to protein stabilization was presented. *Carbohydrate Research* 323, 163-175 (2000).

Artursson et al. demonstrated that proteins could be incorporated into polyacryl starch microparticles. One incorporated protein, the enzyme carbonic anhydrase, retained a low amount of activity at temperatures where the free protein had no activity (e.g., >70°C). At lower temperatures (e.g., < 65°C), however, the free enzyme was

more stable than the enzyme incorporated into the microparticles. *Journal of Pharmaceutical Sciences* 73, 1507-1513 (1984).

Gliko-Kabir et al. demonstrated that the swelling of lyophilized guar gum powder in gastric or intestinal buffer could be reduced from approximately 100 fold to approximately 5 fold if the guar was crosslinked with glutaraldehyde. No data concerning protein stabilization was presented. *Pharmaceutical Research* 15, 1019-1025 (1998).

Bauman et al. demonstrated that carrageenan gum stabilized the enzyme cholinesterase against heat when the enzyme was dried on a urethane foam sheet with 8% starch. *Analytical Biochemistry* 19, 587-592, (1967).

U.S. Pat. No. 6,391,296 B1 issued to Toray Industries, Inc. on May 21, 2002 and European Patent Application No. EP 0 950 663 A1 submitted by the same company and published on October 20, 1999 disclose the use of gum arabic as a protein stabilizer. Aqueous solutions containing 0.2% - 2% gum arabic were shown in these documents to stabilize proteins to storage at 4°C and to freeze drying. There was no data and no discussion concerning the ability of gum arabic to stabilize proteins at room temperature or higher temperatures. While the examples cite the use of gum arabic at 0.2% - 2%, the US patent claims the use of gum arabic from 0.2% to 10%. No mention is made of possible benefits to using gum arabic as a stabilizer at concentrations greater than 10% or at physiological temperature, pH, or salt concentration.

Many of the methods that are known to stabilize proteins, require that the protein be covalently attached to a solid support or covalently substituted with a stabilizing molecule. Covalent modification is not always practical for proteins in solutions and can change the biological effectiveness of a therapeutic protein., Thus there is a need for a protein stabilization system that does not require covalent modification of the protein.

The typical method of administering therapeutic proteins to a patient or test subject is by means of needle-based injections. Currently, many pharmaceutical and drug delivery companies are seeking to develop alternative systems for the delivery of therapeutic proteins. These alternative systems are expected to require fewer dosings and to allow for more effective control over the rate of protein release in the body.

One alternative drug delivery system known in the art includes the formulation of the protein in a biodegradable, water insoluble, polymer matrix. The polymer (e.g., poly(lactic-co-glycolic acid)) can be formulated as an injectable or respirable microparticle. Alternately, the protein can be formulated in a temperature sensitive polymer that is liquid at room temperature but solidifies at 37°C after injection into a patient. A third alternative is for the polymer to be dissolved in a non-toxic water miscible solvent that dissolves in plasma after injection leading to precipitation of the polymer. In all cases, the polymer systems are developed for sustained release of protein over time; however, the stability of the protein during the release period is difficult to maintain and generally less than 50% of the total protein load can be delivered. Additionally, the delivery of the protein is not uniform, but rather occurs with a rapid initial burst which is followed by a much slower rate of sustained protein release.

A second type of known delivery system includes an implanted pump such as an osmotic pump. In this system, a suspension of protein in a water miscible organic solvent is continuously delivered to the patient or test subject through an orifice in the osmotic pump implant. However, use of this system may prove problematic because it is often difficult to suspend a high protein load in the organic solvent, and only some proteins are stable to prolonged incubation under the required non-aqueous or mixed organic-aqueous conditions.

Thus, given the current state of the art, there is a need for compositions and methods that effectively stabilize a variety of proteins in various chemical and physical environments, and that are compatible with a variety of drug delivery systems.

SUMMARY OF THE INVENTION

The present invention is directed to stable aqueous solutions and gels of biologically active proteins wherein the protein solutions and gels are stabilized by high molecular weight polysaccharide gums. The stable protein solutions and gels may be used in drug delivery systems and are protected against stresses such as high temperatures, oxidation, organic solvents, extremes of pH, drying, freezing, and

agitation. Preferably, in the solutions and gels of the invention, the polysaccharide gums are not bound to the protein.

According to a preferred embodiment, the aqueous solutions or gels of the invention include at least one biologically active protein, wherein the protein may be an enzyme, antibody, hormone, growth factor, or cytokine and at least one polysaccharide gum for stabilizing the protein, wherein the polysaccharide gum may be, for example, gum arabic, guar gum, xanthan gum, locust bean gum, gum ghatti, gum karaya, tragacanth gum or a related polysaccharide.

Drug delivery systems compatible with the present invention include implanted subcutaneous delivery systems and intravenous drug delivery systems that can actively or passively deliver the biologically active proteins.

In one embodiment of the present invention, high molecular weight polysaccharide gums are used to stabilize therapeutic proteins delivered by means of implanted drug delivery devices such as a capsule, wherein the capsule includes a molecular weight cut-off membrane with uniform pore size. The polysaccharide gum stabilizes the protein contained by the capsule and the release of the protein can be controlled by the membrane which is permeable to the therapeutic protein but impermeable to the higher molecular weight gum. This embodiment, therefore, would not necessarily be compatible with small molecular weight stabilizers that would diffuse out of the capsule faster than the protein. The membrane retains the polysaccharide gum in the capsule and the capsule prevents the gum from swelling and decreasing in concentration.

BRIEF DESCRIPTION OF THE FIGURES

Figure 1 shows the ability of gum arabic to stabilize interferon-γ under physiological conditions over 4 weeks.

Figure 2 shows the ability of gum arabic to stabilize lactate dehydrogenase (LDH) against oxidative stress by copper and ascorbic acid.

Figure 3 shows the ability of gum arabic to stabilize lactate dehydrogenase against oxidative stress by copper, ascorbic acid, and hydrogen peroxide.

Figure 4 is a schematic diagram of a capsule with a semi-permeable membrane.

Figure 5 is a tabular presentation of chymotrypsin release in 48 hours through a 100K membrane.

Figure 6 is a schematic diagram of the apparatus used to generate the chymotrypsin data for Figure 5.

DETAILED DESCRIPTION OF THE INVENTION

The present invention is directed to a heat stable aqueous solution or gel comprising a biologically effective amount of a protein and a stabilizing effective amount of a polysaccharide gum material. The invention is further directed to a heat stable aqueous solution or gel comprising a biologically effective amount of a protein and a stabilizing effective amount of a polysaccharide gum material wherein said protein is selected from the group consisting of an enzyme, an antibody, a hormone, a growth factor, and a cytokine wherein said gum is selected from the group consisting of gum arabic, guar gum, xanthan gum, locust bean gum, gum karaya, gum ghatti, and tragacanth gum.

Another embodiment of the invention relates to a heat stable solution or gel comprising a pharmaceutically effective amount of a protein and a stabilizing effective amount of a gum material wherein said stabilized solution or gel is contained in an implantable drug delivery device.

A further embodiment of the invention is directed to a lyophilized composition having biological activity, wherein said lyophilized composition is formed by lyophilizing a heat stable solution or gel comprising a biologically effective amount of a protein and a stabilizing effective amount of a gum material. In a preferred embodiment, the lyophilized dry powder is reconstituted in an aqueous buffer to give a high concentration

of gum, wherein the gum preferentially stabilizes the protein to various stresses under physiological conditions. As used herein the term "biologically active protein" includes proteins and polypeptides that are administered to patients as the active drug substance for prevention of or treatment of a disease or condition as well as proteins and polypeptides that are used for diagnostic purposes, such as enzymes used in diagnostic tests or in *in vitro* assays as well as proteins that are administered to a patient to prevent a disease such as a vaccine. Contemplated for use in the compositions of the invention, but not limited to, are therapeutic proteins and polypeptides such as enzymes, e.g., glucocerebrosidase, adenosine deaminase; antibodies, e.g., Herceptin® (trastuzumab), Orthoclone OKT®3 (muromonab- CD3); hormones, e.g., insulin and human growth hormone (HGH); growth factors, e.g., fibroblast growth factor (FGF), nerve growth factor (NGF), human growth hormone releasing factor (HGHRF), and cytokines, e.g., leukemia inhibitory factor (LIF), granulocyte-colony stimulating factor (G-CSF), granulocytemacrophage-colony stimulating factor (GM-CSF), interleukin-6 (IL-6), interleukin-11 (IL-11), interleukin-9 (IL-9), oncostatin-M (OSM), ciliaryneurotrophic factor (CNTF), interferon-α, and interferon-γ.. As used herein, the Polysaccharide Solubility Limit is the concentration of polysaccharide obtained after an aqueous buffer, typically a phosphate buffered saline (PBS) is slowly added to a solid polysaccharide, with thorough mixing, until all of the solid material has either dissolved or has hydrated to form a gel. Depending on the polysaccharide used, the solubility limit can be in the vicinity of 10% or can be higher than 50%. Physiological condition as pertained to this invention is typically human body temperature under normal conditions, that is, 37°C a neutral pH of around 7 ± 1, and a physiological concentration of saline (0.9%).

The term "pharmaceutically effective amount" refers to that amount of a therapeutic protein having a therapeutically relevant effect on a disease or condition to be treated. A therapeutically relevant effect relieves to some extent one or more symptoms of a disease or condition in a patient or returns to normal either partially or completely one or more physiological or biochemical parameters associated with or causative of the disease or condition. Specific details of the dosage of a particular

active protein drug may be found in the drug labeling, i.e., the package insert (see 21 CFR § 201.56 & 201.57) approved by the United States Food and Drug Administration.

Polysaccharide gums are natural products extracted from various plants, trees and bacteria, such as *Cyamopsis tetragonolobus* (guar gum) and *Ceratonia siliqua* (locust bean or carob gum) and *Astragalus gummifer* (tragacanth) from plants of the *Leguminosae* family; gum arabic and tamarind gum from respectively the *Acacia senegal* tree and *Tamarindus indica* tree; xanthan gum from the bacterial genus *Xanthamonas campestris;* gum ghatti from *Anogeissus latifolia* and gum karaya from *Sterculia urens*. Many grades and forms of polysaccharide gums are commercially available.

The gum Arabic used in the solutions of the invention has a highly branched galactose core with linkages to other sugars and contains ~1% glycoprotein; the locust gum used herein has a mannan chain (1->4) with galactose substituted at the 6-position of ~20% of the mannose units; the guar gum used herein has a mannan chain (1->4) with galactose substituted at the 6- position of ~40% of the mannose units; and the xanthan gum used herein has a glucan (1->4) chain with trisaccharides substituted on every other glucose.

According to the preferred embodiment of the present invention, increasing concentrations of high molecular weight polysaccharide gums (i.e., greater than 200 kilodaltons) are utilized for effective protein stabilization. The polysaccharide gums described herein are more effective protein stabilizers than commonly used small molecule protein stabilizers such as monosaccharides, disaccharides, and detergents. High molecular weight, branched chain or substituted polysaccharides such as gum arabic, guar gum, xanthan gum, and locust bean gum are more effective protein stabilizers than linear chain polysaccharides such as cellulose, agarose, xylan, konjak, or chitosan. The polysaccharide gums used herein are typically used at concentrations of gum (% w/v) that are near or at the upper limit of the solubility of the particular gum in aqueous solutions. The gums used herein will form either a viscous solution in water or will form a gel. In general, from about 0.5% to from about 35% weight to volume ("w/v") will be used depending on the particular polysaccharide. Gum arabic has exceptional solubility in aqueous solution and up to about 50% formulations have been made.

Preferred are gum arabic concentrations above 10%, concentrations above 20% give are more preferred, while concentrations above 30% are yet more preferred, and concentrations above 40% are the most preferred as these have resulted in the greatest stability in many of the tests herein. In a preferred embodiment, stabilizing gum concentrations should provide stabilities above 70% protein activity under physiological conditions (at 37°C) for at least about a two weeks and most preferably, for patient ease of use, at least 1 month or more. Recent tests have shown that gum arabic concentrations of about 50% provide about full interferon-gamma activity after 1 month and that 45% activity was still available after two months at physiogical condtions (refer to Table 9).

Polysaccharide gums are hydrogels that can absorb many times their weight of water. Therefore, it is preferable to restrict the tendency of the gums to swell in order to maintain the high polysaccharide concentrations that effectively stabilize proteins. The high gum concentration can be maintained by enclosing the gels in a capsule with a molecular membrane that is permeable to the protein but impermeable to the higher molecular weight gum. The capsules can be implanted into a patient or test subject for the controlled release of stabilized protein over extended periods. Over time, the protein is steadily released from the capsule thus, decreasing the concentration of protein inside the capsule while the concentration of the stabilizing gum within the capsule remains constant.

The present invention includes polysaccharide gums that are incorporated into drug delivery devices for the purposes of (i) stabilizing proteins and (ii) controlling the rate at which the proteins diffuse from the delivery device. The polysaccharide gums of the present invention stabilize native protein conformations, even at high protein concentrations. Thus, the delivery device can be loaded with a protein/gum composition that contains a high concentration of protein, or with a mixture in the solid form, thereby increasing the drug load of the device.

In various embodiments, the compositions of the present invention are utilized for the stabilization of proteins during membrane-controlled release from capsules or other devices implanted into a patient or test subject. In this case, the delivery device is designed to prevent the polysaccharide from swelling so that the stabilizing effects of

high polysaccharide concentrations are maintained inside the capsule. Since it is unnecessary for the polysaccharide gums described herein to bind to proteins to effect protein stabilization, proteins can be released from the solution or gel by diffusion. Additionally, the polymeric properties of polysaccharide gums provide an additional mechanism for stabilizing proteins by restricting a protein's molecular mobility.

The physical state of the polysaccharide gums of the present invention depends on the conditions used to prepare the gum solutions. Viscosities can vary several fold for each gum depending on factors such as the mixing rate used to prepare the hydrated gum and whether the gum was heat treated or freeze-thawed. Manipulating the viscosity of the gums will permit the rate at which proteins are released from the gums to be controlled, as the rate of diffusion is inversely proportional to the solution viscosity.

The stabilized protein solutions and gels of the invention may contain minor amounts (from about 0.5% to about 5.0% w/v) of auxiliaries and/or excipients, such as N-acetyl-dl-tryptophan, caprylate, acetate, citrate, glucose and electrolytes, such as the chlorides, phosphates and bicarbonates of sodium, potassium, calcium and magnesium. They can furthermore contain: acids, bases or buffer substances for adjusting the pH, salts, sugars or polyhydric alcohols for isotonicity and adjustment, preservatives, such as benzyl alcohol or chlorobutanol, and antioxidants, such as sulphites, acetylcysteine, Vitamin E or ascorbic acid.

Suitable tonicity adjustment agents may be, for instance, physiologically acceptable inorganic chlorides, e.g. sodium chloride; sugars such as dextrose; lactose; mannitol; sorbitol and the like. Preservatives suitable for physiological administration may be, for instance, esters of parahydroxybenzoic acid (e.g., methyl, ethyl, propyl and butyl esters, or mixtures of them), chlorocresol and the like.

The pH of the solution can be adjusted using a physiologically acceptable acid e.g. an inorganic mineral acid such as hydrochloric, hydrobromic, sulfuric, phosphoric, nitric and the like, or an organic acid such as acetic, succinic, tartaric, ascorbic, citric, glutamic, benzoic, methanesulphonic, ethanesulfonic and the like, or a physiologically acceptable base, such as sodium hydroxide, potassium hydroxide, calcium hydroxide,

magnesium hydroxide and the like, an physiologically acceptable buffer solution, e.g. a chloride buffer, an acetate buffer, a phosphate buffer and the like.

In another embodiment of the present invention, the drug delivery device is a capsule that is filled with multiple layers of polysaccharide gums of varying viscosities. This capsule includes a molecular membrane capable of retaining the gum, but which is permeable to various therapeutic proteins. In this embodiment, a layer of viscous gum (e.g., guar gum) adjacent to the polymer membrane controls the release of the protein, while a layer of less viscous gum (e.g., gum arabic) that has been formulated with the protein provides a stable reservoir of protein.

In still another embodiment, hollow fibers with specifically defined molecular weight cutoffs are filled with solutions or gels of gum and protein. Hollow fibers with controlled pore sizes are useful for rapidly dialyzing proteins. Preferably, the fibers are made of biocompatible materials that can be implanted in a patient or test subject. The fibers may be filled with solutions of protein formulated with guar gum or with locust gum. The gums control the rate of protein release as well as providing protein stabilization during release. Because the hollow fibers have a very large surface area to volume ratio, this approach is most useful for gum/protein gels with slow protein diffusion rates. A positive attribute of an implant that contains multiple hollow fibers is that all the therapeutic protein will not be in the same capsule, thereby lessening the possibility of a capsule failure, which might release a toxic dose of the protein.

An alternate embodiment of hollow fibers includes the steps of filling the hollow fibers with a gum arabic/protein solution and imbedding multiple fibers in a matrix of guar or locust gums. The guar or locust matrix is then enclosed in a dialysis membrane or a membrane enclosed capsule. This embodiment has many of the stabilization and diffusion properties of the multi-layered capsule approach, but in this case the main drug load is not in a single capsule and is, therefore, less vulnerable to a single capsule failure.

According to the present invention, a preferred method for stabilizing a therapeutic protein in a drug delivery system comprises the steps of (a) providing a protein as an aqueous solution; (b) adding a polysaccharide gum to the protein; and (c) adding the gum/protein solution or gel to a capsule that contains a molecular

membrane. Alternatively, the protein/polysaccharide gum can be dried by lyophilization or spray drying and added to the capsule. In this method, the capsule is preferably fabricated from a biocompatible material and capable of containing the polysaccharide gum in a fixed volume to prevent the gum from swelling upon exposure to an aqueous environment. The capsules comprise a single large protein reservoir or may be comprised of a plurality of hollow fibers. The membrane is fabricated from silica or a polymer and has pore sizes, which permit the membrane to be permeable to the protein but impermeable to the higher molecular weight polysaccharide gum. The polysaccharide gum is selected based on its ability to both stabilize the protein and control the protein's rate of release. In this method, multiple gums and multiple layers of gums can be used in the capsule.

The following examples illustrate the effectiveness of the compositions and methods of the present invention in stabilizing proteins under different environmental conditions and in different model delivery systems.

EXAMPLE 1

A 1 mg/ml solution of chymotrypsin was made by dissolving 20 mgs of chymotrypsin in 20 mL of phosphate buffered saline (PBS, pH 7.4). Solutions of the gums containing chymotrypsin were made as follows: 1.66 ml of 1 mg/mL chymotrypsin solution in PBS was added to 0.83 g of gum arabic and homogenized to give a 33% gum arabic solution by weight, using the assumption that 1 mL of the chymotrypsin/PBS solution will approximately be equal to1 gram. A similar procedure was repeated for 33% sorbitol. To 0.5 g of tragacanth gum, gum guar or xanthan gum, 2 ml of 1 mg/mL chymotrypsin in PBS was added and homogenized to give a 20% solution. To 0.625g of gum karaya and gum ghatti, 1.875mL of 1mg/mL chymotrypsin in PBS was added and homogenized to give a 25% solution. Similarly, to 0.35 g of locust bean gum, 2.15 mL of 1 mg/mL chymotrypsin in PBS was added and homogenized to give a 14% solution. The pH of all the solutions, except 33% sorbitol, was adjusted to 7.4 using 1M NaOH. All the samples (33% gum arabic, 33% sorbitol, 20% tragacanth gum, 20% gum guar, 20% xanthan gum and 14% locust bean gum) were prepared in 50 mL centrifuge

tubes and incubated at 60°C for 7.5 min in a water bath. The samples were cooled on ice and diluted 20-fold in order to give a final concentration of 0.05 mg/mL chymotrypsin. Dilutions were performed by adding 31.73 mL PBS to the 33% gum solutions, 35.625mL for the 25% gum solutions, 38 ml PBS for the 20% gum solutions and 40.85 mL PBS to the 14% gum solution. These solutions were homogenized before assaying them for chymotrypsin activity using N-benzoyl L-tyrosine ethyl ester as the enzyme substrate according to published literature (J. Biotech, 1994, v35, p9-18). The results are summarized in Table 1.

Table 1

The Effect of Polysaccharides on the Stability of the Enzyme Chymotrypsin Incubated at 60°C for 7.5 Minutes

Stabilizer	Concentration of Stabilizer (%w/v)	% Recovery 60°C	% Recovery Room Temp.
None	—	0%	100%
Sorbitol	33%	~27%	~90%
Gum Arabic	33%	~98%	~100%
Locust Gum	14%	~85%	~100%
Guar Gum	20%	~92%	~100%
Xanthan Gum	20%	~50%	~100%
Tragacanth gum	20%	~68%	~100%
Gum Karaya	25%	~30%	~75%
Gum Ghatti	25%	~30%	~100%

As shown in Table 1, an accelerated aging study performed at 60^{0}C , gum arabic, guar gum, xanthan gum, locust bean gum and tragacanth gum all stabilized the activity of the enzyme chymotrypsin over 50%. Recoveries are significantly higher than those obtained by incubation with sorbitol, a monosaccharide shown in the literature to stabilize chymotrypsin. Other gums such as gum karaya and gum ghatti, stabilized chymotrypsin activity around 30%.

As indicated in Example 1, chymotrypsin is stabilized at 60 °C by high concentrations of gum arabic, guar gum, xanthan gum, tragacanth, gum karaya, gum ghatti and locust gum. The stabilizing effects of gum arabic, xanthan gum, locust gum, and guar gum were found to decrease as the gum concentration decreased, as shown in Table 2.

Table 2

The Effect of Different Polysaccharide Concentrations on the Stability of the Enzyme Chymotrypsin Incubated at 60^{0}C for 7.5 Minutes

Gum	% Recovery of Chymotrypsin Activity[a]						
	30% Gum	20% Gum	15% Gum	10% Gum	5% Gum	2.5% Gum	1% Gum
Arabic	92%	ND	38%	ND	ND	ND	ND
Guar	ND	58%	33%	27%	12%	4%	0%
Xanthan	ND	46%	31%	26%	14%	3%	0%
Locust	ND	ND	70%[b]	48%	26%	24%	14%

[a] ND means that the experiment under those conditions was not performed.

[b] Locust gum was tested at 14% rather than 15%.

Following the procedure of Example 1, other polysaccharides and two surfactants were tested. None of the materials listed in Table 3 were effective to stabilize aqueous solutions of chymotrypsin against elevated temperatures.

Table 3

Polysaccharides and Surfactants that do not Stabilize Chymotrypsin at 60°C

Additive	Concentration of Sugar or Surfactant (%w/v)	Structure
Cellulose	25%	Linear β1,4-glucose chain
Agarose	14%	Linear chain of galactose and anhydro-galactose
Beechwood Xylan	50%	Linear xylose chain
Barley Beta Glucan	17%	β1,3-glucan chain
Konjak Glucomannan	25%	Linear chain of glucose + mannose
Chitosan	17%	Linear chain of anhydro-N-acetyl glucosamine
Amylopectin	33%	Branched glucose chain
Untreated Starch	33%	Branched amylopectin + linear amylose
Hydroxyethylstarch	33%	Starch chemically modified with hydroxyethyl groups
Dextran	33%	α 1,6-anhydro-D-glucose chain
Tween 20	1%	Polyoxyethylene(20)sorbitan monolaurate
Tween 80	1%	Polyoxyethylene(20)sorbitan monooleate

Following the procedures described in Example 1, the concentration of chymotrypsin in the solution was varied and the concentration of gum Arabic was held at 33% (w/v) in the solution. The results are summarized in Table 4.

Table 4

Stabilization of Increasing Concentrations Of Chymotrypsin by Gum Arabic

Gum Arabic Concentration (% w/v)	Chymotrypsin Concentration (mg/ml)	% Activity 7.5 Min., 60°C
0	1.0	0
0	3.3	0
0	10.	0
0	33.	0
0	100	0
33%	1	97 ± 17%[a]
33%	3.3	62 ± 6%[a]
33%	10	57 ± 5%[a]
33%	33	41%[b]
33%	100	53%[b]

[a] Average result of two experiments [b] Result of a single experiment

EXAMPLE 2

Gum arabic (33%)chymotrypsin solutions were prepared as described in Example 1, with the exception that PBS was made with 0.1% sodium azide. A solution containing 1mg/ml chymotrypsin and no gum arabic was prepared as the control. Aliquots of the test and control solutions were added to centrifuge tubes and these tubes were incubated at 37^{0}C for a period of time. The results are summarized in Table 5.

Table 5

Chymotrypsin Stabilization at 37^0 C by 33%(w/v) Gum Arabic

Stabilizer	Time Weeks	% Activity
Control (None)	0	100
Control	1	15
Control	2	0
Control	4	0
Gum Arabic	1	130
Gum Arabic	2	80
Gum Arabic	4	82
Gum Arabic	8	58

As shown in Table 5, gum arabic was tested for its ability to stabilize chymotrypsin to long-term incubation at 37^{0}C in aqueous buffer, pH 7.4 (physiological conditions). The results shown in Table 5 indicate that gum arabic protects chymotrypsin and only approximately 40% of the activity of chymotrypsin is lost after incubation at 37^{0}C for eight weeks. In contrast, 85% of the activity is lost after one week and all the activity is lost after the second week at 37^{0}C in the absence of stabilizer.

It is important that the concentration of the gum stabilizer in the aqueous solution be high enough to effectively stabilize the protein. The stabilization of the particular protein is dependent on the concentration of polysaccharide gum in the solution. As shown in Table 6, the activity of chymotrypsin exposed to heat stress depends on the concentration of gum, with higher concentrations giving better stability.

Table 6

High Gum Concentrations are Required for Optimal Protein Stabilization

Concentration (%w/v) Of Gum Arabic	% Activity Recovered 1 Week, 37^0 C
None	0
33	73
20	85
10	62
5	43
2.5	21
1.0	17

As indicated in Table 6, gum arabic is a stabilizer of chymotrypsin from 1% to 33%. guar gum has been found to provide stabilization to chymotrypsin at a concentration of about 2.5 to 20% (w/v), xanthan gum and tragacanth gum provide stabilization at a concentrations of about 1.5 to 20% (w/v), gum karaya and gum ghatti are effective in the concentration range of 1 to 25% and locust gum is effective at a concentration of about 1 to 14% (w/v). However, concentrations of gum arabic typically above 10% (w/v) are required for effective use in medical and industrial settings. Typically from one to several weeks, or months of stability are preferred.

The polysaccharide gum stabilizers described herein, not only are able to stabilize proteins in solution but are also able to protect such proteins through conventional lyophilization and subsequent reconstitution. In a preferred application,

the reconstitution of a lyophilized mixture of gum and protein is carried out in a manner as to maintain the high concentrations of the gum. Example 3, describes the preparation of an aqueous solution of the enzyme lactate dehydrogenase (LDH), an enzyme that loses its activity when lyophilized and then reconstituted.

EXAMPLE 3

A 1 mg/mL solution of lactate dehydrogenase (LDH) was prepared by dissolving 5mgs LDH in 5 mL of PBS, pH 7.4. A 10 μg/mL stock solution of LDH was prepared by dissolving 200 μL of 1 mg/mL LDH solution in 19.8 mL PBS. The gum solutions with LDH were prepared as follows: to 0.25 g of each gum or sorbitol, 2.25 mL of 10 μg/mL LDH was added and homogenized. Aliquots of 1 mL in plastic Eppendorf tubes were frozen at –70°C for 30 min and lyophilized overnight using a Labconco model 77530 lyophilizer. To each tube of the LDH lyophilizate, 2.25 mL water was added to give a reconstituted solution of 10 μg/ml enzyme. The reconstituted lactate dehydrogenase was assayed for activity using the published literature method of Lovell and Winzor (Biochemistry, 1974, v13, 3527). The results are summarized in Table 7.

Table 7

Stabilization of Lyophilized Lactate Dehydrogenase (LDH)[a]

Stabilizer	Concentration Before Lyophilization	% Activity Recovered
None	10	20
Sorbitol	10	20
Gum Arabic	10	64
Xanthan gum	10	37
Locust Bean Gum	10	93
Agarose	10	59
Guar Gum	10	78

[a] the "% Activity" was not corrected for the extraction yield of LDH from the gum solution.

As shown in Table 7, polysaccharide gums were tested for their ability to stabilize lactate dehydrogenase, an enzyme that loses activity when lyophilized. Both the gums that were effective thermal stabilizers (i.e., gum arabic, guar gum, xanthan gum, locust bean gum) and a gum that provided no thermal stabilization (i.e., agarose) were effective stabilizers of lactate dehydrogenase during the lyophilization process. This result indicates that polysaccharide gums are effective stabilizers of a lyophilized protein and shows that the gums can be used to protect therapeutic proteins that are lyophilized prior to their addition to a drug delivery device. The protein/polysaccharide powder may be added to the delivery device dry, as a solution, as a gel, or as slurry. The lyophilized protein-polysaccharide gums powder will be useful for long term shelf storage of therapeutic proteins as well. In another embodiment, the lyophilized powder will be stable in aqueous buffer solution when it is reconstituted if a high enough concentration of polysaccharide gum is present in the lyophilized powder or if an additional amount is added later. Preferably the lyophilized powder will contain a sufficient concentration of gum to stabilize the material before and after reconstitution.

Example 4

Following the procedure described in Example 1, solutions of lysozyme (1 mg/mL), lactate dehydrogenase (50 μg/mL), and glucose-6-phosphate dehydrogenase (50 μg/mL) were subjected to accelerated aging at respectively 90°C, 60^0C, and 50^0C, and for 10 minutes each. The activity of lactate dehydrogenase was assayed in accordance with the procedure of Lovell and Winzor (Biochemistry, 1974, v13, 3527) described in Example 3. The activity of glucose-6-phosphate dehydrogenase was assayed using the method published in Arch. Biochem. Biophys, 1998, v360, p10-14. The activity of lysozyme was assayed according to the method published in Biochimica et Biophysica Acta, 1952, v8, p 302 – 309. The results are summarized in Table 8.

Table 8

Thermal Stabilization of Lactate Dehydrogenase ,Glucose-6-Phosphate Dehydrogenase, and Lysozyme By Polysaccharide Gums

Protein	Conc. of Protein	Stabilizer	Conc. of Stabilizer (%w/v)	Accelerated Aging Conditions	% Activity
Lysozyme	1 mg/mL	None	0	90°C; 10 min	5
Lysozyme	1 mg/mL	Gum Arabic	33	90°C; 10 min	85
Lactate Dehydro-genase	50µg/mL	None	0	60° C; 10 min	2
Lactate Dehydro-genase	50µg/mL	Trehalose	20	60° C; 10 min	2
Lactate Dehydro-genase	50µg/mL	Gum Arabic	33	60° C; 10 min	23
Glucose-6-phosphate dehydro-genase	50µg/mL	None	0	50° C; 10 min	0
Glucose-6-phosphate dehydro-genase	50µg/mL	Gum Arabic	33	50° C; 10 min	5
Glucose-6-phosphate dehydro-genase	50µg/mL	Guar Gum	20	50° C; 10 min	60
Glucose-6-phosphate dehydro-genase	50µg/mL	Trehalose	20	50° C; 10 min	20

As shown in Table 8, various proteins are stabilized against accelerated heat stress by different polysaccharide gums. In a specific example, while glucose-6-phosphate dehydrogenase is not stabilized significantly by gum Arabic, another polysaccharide, gum guar provides good stability. Thus it is possible to stabilize different proteins using different polysaccharides. Thus, in a general embodiment, the stabilization of various proteins by polysaccharides is not limited to the ones given in the above examples.

Example 5

Commercial gum arabic was purified by a sequence of dialysis, filtration, and lyophilization. Dialysis was performed by first making a 9% solution of gum arabic in deionized water, homogenizing the sample, and then adjusting the pH to 7.4 with 1 M sodium hydroxide. The solution was put into 10,000 molecular weight cut-off dialysis tubing and dialyzed at 4°C twice against PBS and twice against deionized water. The sample was then centrifuged and the supernatant filtered through a 0.22 μm filter, and lyophilized.

Example 6

Following the procedures described in Example 1, aliquots of interferon-γ (0.02 mg/mL) were incubated at 37°C in PBS with 0.1% azide that contained no stabilizers, 33% purified gum arabic, and 50% purified gum arabic (described in Example 5). Following incubation, samples were diluted in PBS that contained 0.5% BSA and 0.05% Tween 20. The activity of the interferon-γ in the sample was then measured using an enzyme linked immunosorbent assay that has been shown to correspond to the bioactivity (Mechanisms of Ageing and Development, 121, 47 – 58 (2000). The data is presented in Table 9 and Figure 1.

Table 9

Thermal Stabilization of Interferon-γ By Gum Arabic at 37°C and pH 7.4

Protein + Gum Arabic (GA)	Activity 1 Week	Activity 2 Weeks	Activity 4 Weeks
Interferon-γ + 33% GA[a]	106 ± 17%	62 ± 15%	40 ± 13%
Interferon-γ + 50% GA[b]	133%	97%	133%
Interferon-γ Control[b] (no stabilizers)	1%	1%	9%

[a] These results are from the average of three separate experiments, each using duplicate samples.

[b] These results are from a single experiment using duplicate samples.

Example 7

This example illustrates the ability of gum arabic to stabilize proteins against metal catalyzed oxidation (MCO) reactions. Lactate dehydrogenase is a tetrameric enzyme that catalyzes the conversion of pyruvate to lactate in the presence of NADH. The enzymatic activity of LDH is affected in the presence of oxidants and is catalyzed by the presence of trace amounts of metal ions in buffers. LDH, 10^{-5}mM or 1.4μg/ml, was prepared in 80mM phosphate buffer containing 0.2M potassium chloride. MCO reactions were carried out with 0.04mM Cupric Sulfate, 2mM ascorbic acid (Asc) in the presence or absence of 2mM hydrogen peroxide (H_2O_2). All reactions were initiated by the addition of the reductant (ascorbic acid) or oxidant (hydrogen peroxide). The reaction mixture was incubated at room temperature for 15 minutes and assayed using the following protocol for a 96-well plate reader. A 2.64mM NADH and 4mM Sodium pyruvate stock solutions were made in 0.1M phosphate buffer, pH 7.3. All LDH

solutions were diluted to 0.5ug/ml from the MCO reactions. Two hundred µl LDH and 25µl NADH were mixed in a microtiter plate. The reaction was initiated by adding 75µl of sodium pyruvate and decrease in absorbance at 340nm was monitored for 10 minutes. The results are tabulated in Table 10 and shown in Figures 2 and 3. Figure 2 illustrates the stabilization of lactate dehydrogenase (LDH) against oxidative stress caused by copper (Cu^{2+}) + ascorbic acid (Asc) with different concentrations of gum arabic (GA). Figure 3 illustrates the stabilization of lactate dehydrogenase (LDH) against oxidative stress caused by copper (Cu^{2+}) + ascorbic acid (Asc) + hydrogen peroxide (H_2O_2) with different concentrations of gum arabic (GA).

Table 10

Stabilizing Lactate dehydrogenase (LDH) against Oxidative Stress.

Reaction Conditions	% Activity
LDH	100
LDH + Cu^{2+} + Ascorbic Acid	2
LDH + Cu^{2+} + Ascorbic Acid + 50% GA	121
LDH + Cu^{2+} + Ascorbic Acid + 33% GA	75
LDH + Cu^{2+} + Ascorbic Acid + 10% GA	37
LDH + Cu^{2+} + Ascorbic Acid + 2% GA	1
LDH + Cu^{2+} + Ascorbic Acid + H_2O_2	1
LDH + Cu^{2+} + Ascorbic Acid + H_2O_2 + 50% GA	102
LDH + Cu^{2+} + Ascorbic Acid + H_2O_2 + 33% GA	48
LDH + Cu^{2+} + Ascorbic Acid + H_2O_2 + 10% GA	2
LDH + Cu^{2+} + Ascorbic Acid + H_2O_2 + 2% GA	1

As seen in Table 10, gum arabic at 50% is an effective stabilizer against oxidative degradation of chymotrypsin. The stabilization decreases as the concentration of gum arabic decreases.

Referring now to Figure 4, a preferred method for stabilizing a protein used in a drug delivery system includes the steps of providing a protein as an aqueous solution; adding at least one polysaccharide gum to the protein to form an aqueous solution or gel and adding the solution or the gel to a delivery system 40. The delivery system 40 typically comprises a capsule 41, wherein the capsule 41 further comprises a porous semipermeable or molecular membrane 43. The capsule 41 is fabricated from a biocompatible material, and typically contains the protein 45 and polysaccharide gum 47 in a fixed volume, preventing the gum 47 from swelling when exposed to an aqueous environment. The membrane 43 of the capsule 41 is fabricated from silica or a polymer and comprises pores 49 of a size that make the membrane 43 permeable to the proteinaceous materials but impermeable to the larger polysaccharide gum. The rate at which the protein diffuses from the capsule 41 can be controlled by the viscosity of the gum as well as by the permeability of the membrane 43. Further advantages of the present invention will become apparent to those of ordinary skill in the art upon reading and understanding the detailed description herein of the preferred embodiments.

Example 8

This example illustrates chymotrypsin release through a 100K membrane using a 30% gum arabic, 5% gum guar or 5% locust bean gum solution. The release of chymotrypsin from the polysaccharide gums was evaluated because release of proteinaceous materials from the microcapsule is an essential requirement for the gums to be used for protein delivery,. Referring now to Figures 5 and 6, chymotrypsin was dissolved in a solution containing 30% gum arabic and added to capsules 63 that contained 100K molecular weight cut off dialysis membranes. The protein diffuses out of the viscous polysaccharide, through the membrane 67, and into the exchange buffer solution 68 at approximately 85% of the rate at which chymotrypsin diffuses from aqueous buffer through the membrane. The rates at which chymotrypsin diffuses through guar gum and locust bean gum through the membrane 67are approximately

20% of the rate obtained with an aqueous control. This indicates that guar and locust bean gums can be used to significantly reduce the rate of protein delivery through a membrane device, in addition to providing stabilization for the protein. Additionally, mixtures of various gums can effectively be used to stabilize and control the release of therapeutic proteins from implantable capsules.

Figure 6 illustrates the system 60 used for evaluating used chymotrypsin release. A falcon tube 61 was fitted with a capsule 63 containing the chymotrypsin and gum materials 64. A cap 65 having a hole fitted with membrane 67 allowed for diffusion of chymotrypsin into an exchange buffer 68. A magnetic stir bar 69 provided for thorough mixing of the exchange buffer 68.

The invention being thus described, it will be obvious that the same may be varied in many ways. Such variations are not to be regarded as a departure from the spirit and scope of the invention, and all such modifications as would be obvious to one skilled in the art are intended to be included within the scope of the following claims.

What is claimed is:

1. A heat stable aqueous solution or gel comprising a biologically effective amount of a protein and a stabilizing effective amount of a polysaccharide gum material.

2. A heat stable aqueous solution or gel according to claim 1 containing one or more minor amounts of a pharmaceutically acceptable excipient.

3. A heat stable aqueous solution or gel according to claim 1 wherein said protein comprises an enzyme, an antibody, a hormone, a growth factor, and a cytokine.

4. A heat stable aqueous solution or gel according to claim 3 wherein said protein is a hormone.

5. A heat stable aqueous solution or gel according to claim 3 wherein said protein is a cytokine.

6. A heat stable aqueous solution or gel according to claim 1 wherein said gum comprises gum arabic, guar gum, xanthan gum, locust bean gum, tragacanth gum, gum karaya, and gum ghatti.

7. A heat stable aqueous solution or gel according to claim 6 wherein said gum is gum arabic.

8. A heat stable aqueous solution or gel according to claim 6 wherein said gum is present at from about 5%(w/v) to about 50%(w/v) or the gum's solubility limit, and wherein when gum Arabic is selected, the concentration is over 10%(w/v).

9. A heat stable aqueous solution or gel according to claim 8 wherein said gum is present above 10%(w/v) to about 50%(w/v), or the gum's solubility limit.

10. A heat stable aqueous solution or gel according to claim 9 wherein said gum is present at from about 20% (w/v) to about 50% (w/v), or the gum's solubility limit.

11. A heat stable aqueous solution or gel according to claim 2 wherein said pharmaceutically acceptable excipient is selected from the group consisting of antioxidants, preservatives and surface active agents.

12. A heat stable aqueous solution or gel for use in an implantable drug delivery device comprising a pharmaceutically effective amount of a protein and a stabilizing effective amount of a polysaccharide gum material.

13. A heat stable solution or gel according to claim 12 wherein said stabilized solution or gel contains one or more minor amounts of a pharmaceutically acceptable excipient.

14. A heat stable aqueous solution or gel according to claim 13 wherein said pharmaceutically acceptable excipient is selected from the group consisting of antioxidants, preservatives and surface active agents.

15. A heat stable aqueous solution or gel according to claim 12 wherein said protein is selected from the group consisting of an antibody, a hormone, a growth factor, and a cytokine.

16. A heat stable aqueous solution or gel according to claim 15 wherein said protein is a hormone or a growth factor.

17. A heat stable aqueous solution or gel according to claim 15 wherein said protein is a cytokine.

18. A heat stable aqueous solution or gel according to claim 12 wherein said gum is selected from the group consisting of gum arabic, guar gum, xanthan gum, locust bean gum, tragacanth gum, gum karaya, and gum ghatti.

19. A heat stable aqueous solution or gel according to claim 18 wherein said gum is gum arabic.

20. A heat stable aqueous solution or gel according to claim 18 wherein said gum is present at from about 5% (w/v) to about 50% (w/v) or the gum's solubility limit.

21. A heat stable aqueous solution or gel according to claim 20 wherein said gum is present at from about 10% (w/v) to about 50% (w/v) or the gum's solubility limit.

22. A heat stable aqueous solution or gel according to claim 21 wherein said gum is present at from about 20% (w/v) to about 50% (w/v) or the gum's solubility limit

23. A lyophilized composition having biological activity, wherein said lyophilized composition is formed by lyophilizing a heat stable solution or gel comprising a biologically effective amount of a protein and a stabilizing effective amount of a polysaccharide gum material.

24. The lyophilized composition according to Claim 23, wherein the lyophilized polysaccharide and protein dry particles are reconstituted in an aqueous buffer in a manner so as to get high concentration of gum that stabilizes the protein.

25. A lyophilized composition according to claim 23, wherein the said lyophilized powder is added to an implantable device that controls the release of therapeutic protein.

26. A lyophilized composition according to claim 23, wherein the lyophilized powder is reconstituted in an aqueous buffer to provide high concentrations of the polysaccharide that will stabilize the said therapeutic protein, and this reconstituted aqueous gum gel or solution is further added to an implantable device.

27. A lyophilized composition according to claim 23 wherein said protein is selected from the group consisting of an enzyme, an antibody, a hormone, a growth factor, and a cytokine.

28. A lyophilized composition according to claim 23 wherein said gum is selected from the group consisting of gum arabic, guar gum, xanthan gum, locust bean gum, tragacanth gum, gum karaya, and gum ghatti.

29. A lyophilized composition according to claim 28 wherein said gum is gum arabic.

30. A lyophilized composition according to claim 28 wherein said gum is present at from about 5% to about 50% (w/v) after reconstitution in aqueous buffer, or the solubility limit of the gum, and wherein when gum arabic is selected said gum is present at greater than 10% (w/v).

31. A lyophilized composition according to claim 30 wherein said gum is present at from about 10% (w/v) to from about 50% (w/v) after reconstitution in aqueous buffer.

32. A lyophilized composition according to claim 31 wherein said gum is present at from about 20% (w/v) to from about 50% (w/v) after reconstitution in aqueous buffer..

33. A lyophilized composition according to claim 23 optionally containing one or more pharmaceutically acceptable excipients.

34. An implantable drug delivery device containing a heat stable aqueous solution or gel comprising a pharmaceutically effective amount of a protein and a stabilizing effective amount of a polysaccharide gum material.

35. An implantable drug delivery device containing a lyophilized powder according to claim 23.

36. An implantable drug delivery device that is filled with a lyophilized powder according to claim 23 that has been reconstituted with an aqueous buffer to provide a thermally stabilizing amount of polysaccharide and pharmaceutically acceptable amount of therapeutic protein.

37. An implantable drug delivery device according to claim 32 wherein said stabilized solution or gel contains one or more minor amounts of a pharmaceutically acceptable excipient.

38. An implantable drug delivery device according to claim 33 wherein said pharmaceutically acceptable excipient is selected from the group consisting of antioxidants, preservatives and surface active agents.

39. An implantable drug delivery device according to claim 32 wherein said protein is selected from the group consisting of an antibody, a hormone, a growth factor, and a cytokine.

40. An implantable drug delivery device according to claim 35 wherein said protein is a hormone or a growth factor.

41. An implantable drug delivery device according to claim 32 wherein said gum is selected from the group consisting of gum arabic, guar gum, xanthan gum, locust bean gum, tragacanth gum, gum karaya, and gum ghatti.

42. An implantable drug delivery device according to claim 37 wherein said gum is gum arabic.

43. An implantable drug delivery device according to claim 37 wherein said gum is present at from about 5% (w/v) to from about 50% (w/v) or the gum's solubility limit.

44. An implantable drug delivery device according to claim 39 wherein said gum is present at from about 10% (w/v) to about 50% (w/v) or the gum's solubility limit.

45. An implantable drug delivery device according to claim 40 wherein said gum is present at from about 20% (w/v) to about 50% (w/v) or the gum's solubility limit.

46. An implantable drug delivery device according to claim 40 wherein said gum is present at from about 30% (w/v) to about 50% (w/v) or the gum's solubility limit.

47. An implantable drug delivery device according to claim 40 wherein said gum is present at from about 40% (w/v) to about 50% (w/v) or the gum's solubility limit.

48. An aqueous solution or gel stable against metal catalized oxidation reactions comprising a biologically effective amount of a protein and a stabilizing effective amount of a polysaccharide gum material.

49. The oxidation stable solution or gel according to claim 48, wherein when gum arabic is selected said gum is present above about 30%(w/v) to the gums solubility limit.

Figure 1

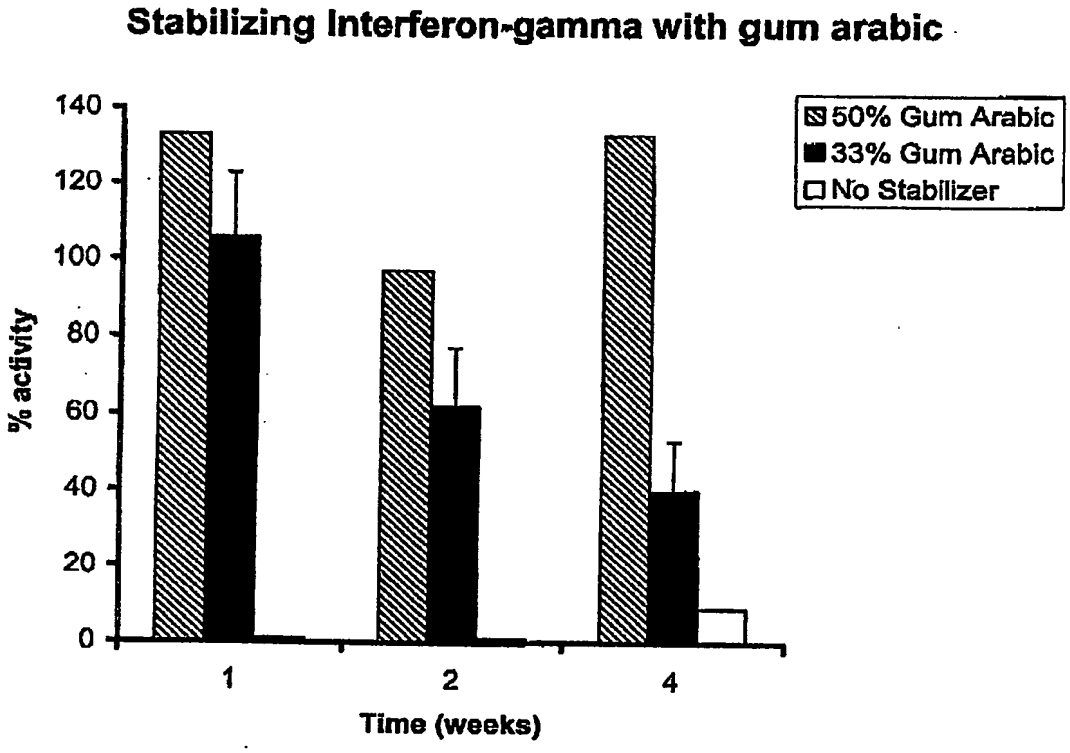
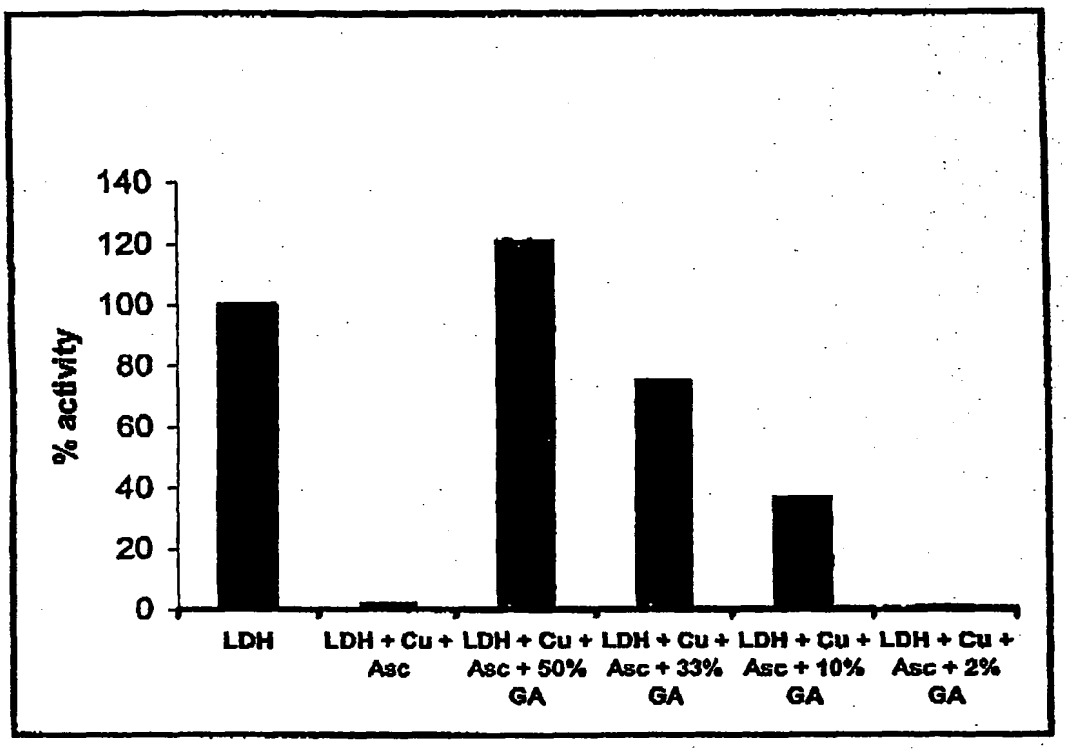

Stabilizing Interferon-gamma with gum arabic
50% Gum Arabic
33% Gum Arabic
No Stabilizer
% activity
140
120
100
80
60
40
20
0
1
2
4
Time (weeks)

Figure 2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LDH
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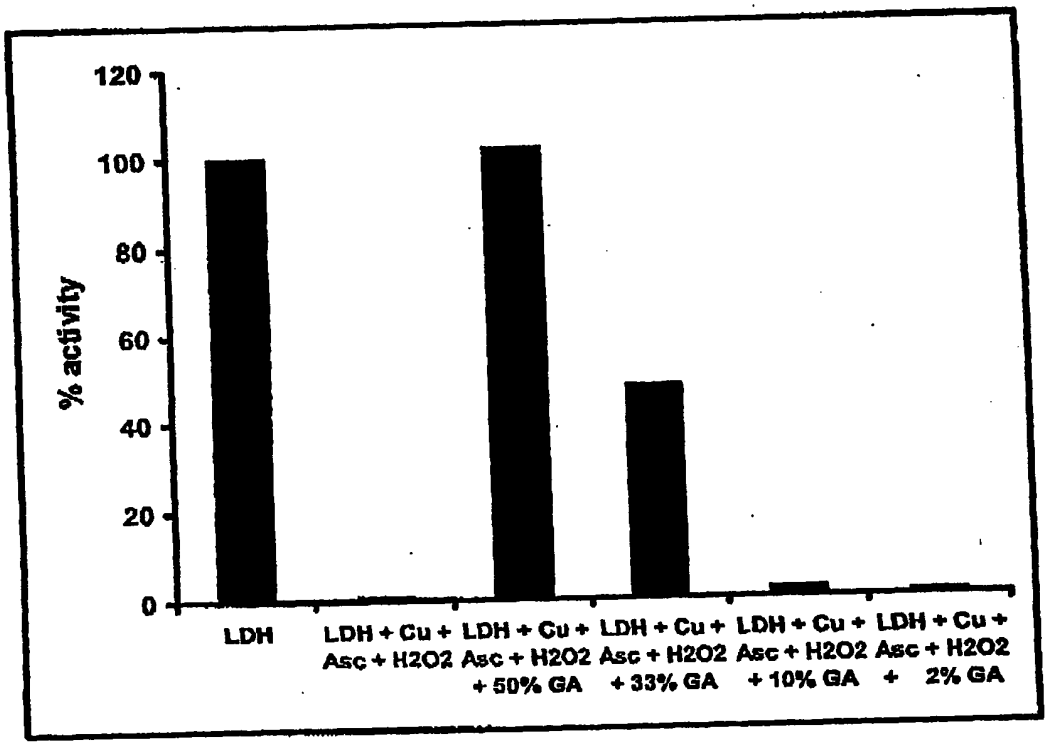
LDH + Cu + Asc + 10% GA
LDH + Cu + Asc + 2% GA

Figure 3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% activity
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LDH + Cu + Asc + H2O2 + 50% GA
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LDH + Cu + Asc + H2O2 + 10% GA
LDH + Cu + Asc + H2O2 + 2% GA

Figure 4

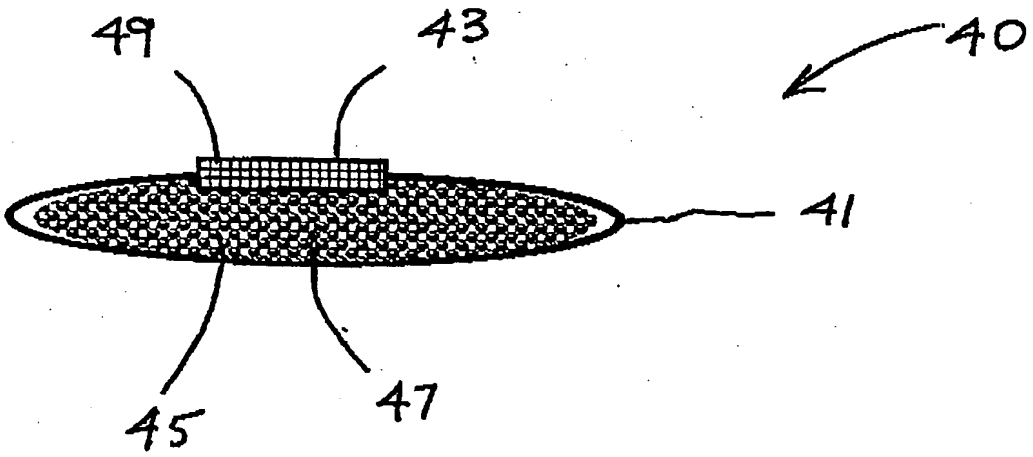


Figure 6

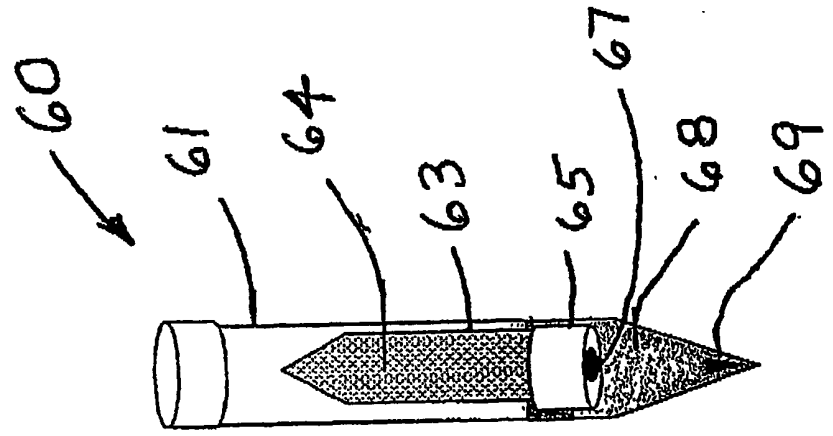
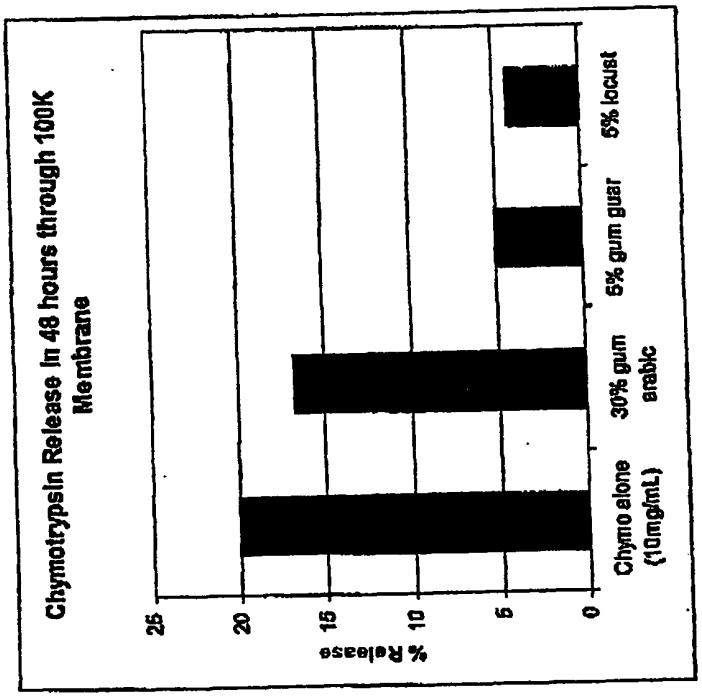


Figure 5



(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
15 May 2003 (15.05.2003)

PCT

(10) International Publication Number
WO 03/040398 A3

(51) International Patent Classification[7]: A61F 2/02, A61K 47/36

(21) International Application Number: PCT/US02/34752

(22) International Filing Date: 30 October 2002 (30.10.2002)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
10/012,667 30 October 2001 (30.10.2001) US

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(81) Designated States *(national)*: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, OM, PH, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZM, ZW.

(84) Designated States *(regional)*: ARIPO patent (GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, SK, TR), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

Published:
— *with international search report*
— *before the expiration of the time limit for amending the claims and to be republished in the event of receipt of amendments*

(88) Date of publication of the international search report:
17 July 2003

For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

WO 03/040398 A3

(54) Title: PROTEINS STABILIZED WITH POLYSACCHARIDE GUMS

(57) Abstract: Described are heat stable aqueous solutions or gels comprising a biologically effective amount of a protein and an effective stabilizing amount of a polysaccharide gum as well as heat stable solutions or gels suitable for use in an implantable drug delivery device at body temperature. Also disclosed are lyophilized compositions having biologically activity, where such lyophilized compositions are formed by lyophilozing the stabilized solutions or gels of the invention. Such lyophilized powders can be used after reconstitution with an amount of aqueous that provide an effective stabilizing concentration of polysaccharide and a pharmaceutically acceptable amount of therapeutic protein particularly against thermal and oxidative stress.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US02/34752

A. CLASSIFICATION OF SUBJECT MATTER

IPC(7) : A61F 2/02; A61K 47/36
US CL : 424/424, 425; 514/780, 782

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
U.S. : 424/424, 425; 514/780, 782

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	US 5,736625 A (CALLSTROM et al) 07 April 1998, see Abstract; column 1, lines 19-30; column 3, lines 8-11, 43-50; column 4, lines 21-39.	1-5, 11-17, 23-27, 33-40, and 48

☐ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

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Date of the actual completion of the international search
20 December 2002 (20.12.2002)

Date of mailing of the international search report
28 MAY 2003

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Form PCT/ISA/210 (second sheet) (July 1998)